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Biophysical Characterization of a *β*-Peptide Bundle: Comparison to Natural Proteins

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We recently described the high-resolution X-ray structure of a helical bundle composed of eight copies of the *β*-peptide Zwit-1F (Figure 1A,B).[1] Like many proteins in Nature, the Zwit-1F octamer contains parallel and antiparallel helices, extensive inter-helical electrostatic interactions, and a solvent-excluded hydrophobic core. Here we explore the stability of the Zwit-1F octamer in solution using circular dichroism (CD) spectroscopy, analytical ultracentrifugation (AU), differential scanning calorimetry (DSC), and NMR. These studies demonstrate that the thermodynamic and kinetic properties of Zwit-1F closely resemble those of natural α-helical bundle proteins.

CD spectroscopy indicates that Zwit-1F is minimally 3_{14}-helical in dilute solution (as judged by the molar residue ellipticity at 205 nm, MRE_{205})[2] but undergoes a large increase in helical structure between 20 and 200 μM (Figure 1C). The concentration dependence of MRE_{205} fits a monomer–octamer equilibrium with an association constant of 4.0×10^{30} M^{-7} ($\ln K_a = 70.5 \pm 1.9$).[3] This value matches the result of AU analysis, which fits a monomer–octamer equilibrium with $\ln K_a = 71.0 \pm 0.9$.[3] Taken together, the AU and CD data support a model in which the unfolded Zwit-1F monomer is in equilibrium with the folded octamer.[4]

Few known natural proteins assemble as octamers. Examples include the histones[5] (hetero-octamer), TATA binding protein[6] (octamer in 1 M KCl), and the well-characterized hemerythrin ($\ln K_a = 84$).[7] Although Zwit-1F is less stable than hemerythrin, it is smaller in mass (13.1 vs 110 kDa) and interaction surface area (7000 vs 15 000 Å^2).[1,8] To compare the stability of Zwit-1F to that of proteins of diverse size and stoichiometry, we calculated the free energy of association per Å^2 of buried surface area (ΔG_{area}). The ΔG_{area} of Zwit-1F is higher than that of hemerythrin, the tetrameric aldolase, and natural helical bundle proteins GCN4 and ROP (Table 1). In fact, ΔG_{area} for Zwit-1F is close to the average value (7.0 ± 2.8 cal·mol^{-1}·A^{-2}) observed for protein complexes burying at least 1000 Å^2 of surface area upon association.[9,10] This comparison implies that the lower affinity of Zwit-1F is due to its small size and not an inherent instability of β^3-peptide complexes.

Temperature-dependent CD studies (Figure 2A) show Zwit-1F to exhibit a concentration-dependent T_m, an inherent property of protein quaternary structure.[14] The Zwit-1F T_m, which increases from 57 °C at 50 μM to 95 °C at 300 μM, is comparable to T_m values of thermostable proteins such as ubiquitin (T_m = 90 °C) and bovine pancreatic trypsin inhibitor (T_m = 101 °C).

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Supporting Information Available: Experimental procedures, Table 1 calculations, and data fits (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

[15] The Zwit-1F T_m is significantly higher than the T_m of GCN4 (41–78 °C at 1–880 μM)[16] and ROP (58–71 °C at 0.5–160 μM).[17] We note, however, that the unfolding of Zwit-1F is less cooperative: the width of the temperature derivative of the CD signal at half-maximum is 40 versus 20 °C for GCN4 or 15 °C for ROP.[16,17]

A high T_m is not a definitive measurement of thermodynamic stability, so DSC was used to further characterize Zwit-1F unfolding (Figure 2B). At 300 μM concentration (where Zwit-1F is 87% octameric), the temperature-dependent heat capacity (C_P) peaks near the T_m identified by CD. This peak is embedded in a sloping baseline ($\partial C_p/\partial T$ = 5.1 cal·mol^{-1}·K^{-2}) 3.1 mcal·g^{-1}·K^{-2}) that is similar to the C_P versus temperature plot of monomeric β^3-peptides, for which no cooperative unfolding peak has yet been observed.[2] For most natural proteins, ($\partial C_p/\partial T$) is about 1 mcal·g^{-1}·K^{-2} in the folded state,[15] but GCN4 ($\partial C_p/\partial T$ = 3.6 mcal·g^{-1}·K^{-2})[16] and some ROP mutants ($\partial C_p/\partial T$ = 4–5 mcal·g^{-1}·K^{-2})[13] have sharply sloped pretransition baselines like Zwit-1F.

The DSC data fit well to a process defined by a two-state transition with dissociation of eight subunits using the program EXAM.[3,18] The fitted enthalpy and heat capacity change per mole octamer are 107.4 ± 0.3 kcal·mol^{-1} and 1.4 ± 0.1 kcal·mol^{-1}·K^{-1}, respectively. Substituting these values into the Gibbs–Helmholz equation[3] yields an equilibrium constant of 5.3 × 10^{31} (ln K = 73.3 ± 1.4) at 25 °C, in excellent agreement with values derived from CD and AU data. The integrated calorimetric unfolding enthalpy (ΔH_{Cal}) for Zwit-1F is 7.2 cal·g^{-1}, within the range observed for natural globular proteins (5.2–11.8 cal·g^{-1}),[19,20] but somewhat lower than GCN4 (7.7 cal·g^{-1})[21] and ROP (9.5 cal·g^{-1}).[17]

The NMR spectra of many well-folded natural and designed proteins are characterized by differentiated amide resonances and slow hydrogen/deuterium exchange.[22] The amide N–H resonances in the ^{1}H spectrum of Zwit-1F, under conditions where the sample is 97% octameric, span 1.4 ppm (Figure 3A). While this span is narrower than that observed in the NMR spectra of large proteins such as α-lactalbumin (3 ppm), it is comparable to that seen for coiled-coil proteins GCN4 and ROP (1.3 and 2.2 ppm, respectively).[13,23,24] In contrast to Zwit-1F, the amide resonances of the poorly folded, monomeric β-peptide Acid-1Y^{A2,11} span only 0.5 ppm.[3] These results indicate that the Zwit-1F fold in solution creates distinct electronic environments for the amide backbone protons.

Participation in a hydrogen bond can protect an amide N–H from exchange with bulk solvent; since exchange occurs from the unfolded state, a slow amide exchange rate constant (k_{ex}) correlates with protein stability in solution.[22] Exchange is often characterized by a protection factor (P) equal to k_{rc}/k_{ex}, where k_{rc} is the rate constant for exchange of a random coil amide N–H under similar conditions. When a lyophilized sample of Zwit-1F is redissolved at 1.5 mM concentration in D_2O, 9 of 14 resolvable peaks require more than 4 h to become indistinguishable from baseline. The time dependence of exchange corresponds to exchange rate constants between 0.6 × 10^{-4} and 2.9 × 10^{-4} s^{-1}. Using β-alanine (βG in our nomenclature) as a random coil model,[3,25] these values of k_{ex} correspond to a protection factor of 2 × 10^4 for Zwit-1F. Thus, amide protons in Zwit-1F are less protected than those in large protein cores, where $P \geq 10^5$.[22,26] However, the protection factor for Zwit-1F, like the span of amide resonances, is comparable to ROP (10^5 at 250 μM)[13] and GCN4 (10^4 at 1.0 mM).[23,24] Acid-1Y^{A2,11} undergoes amide N–H exchange in less than 10 min, showing that slow exchange requires a stable β-peptide fold.[3]

The biophysical experiments presented here describe the thermodynamic and kinetic stability of the Zwit-1F octamer in solution. The data allow us to quantify the similarity of Zwit-1F to GCN4 and ROP, two small, well-folded α-amino acid helix bundle proteins. In fact, the T_m, ΔG_{area}, and ΔH_{Cal} for Zwit-1F are even comparable to much larger natural proteins. Taken

together with the recent high-resolution structure of Zwit-1F,[1] these studies show that β-amino acid heteropolymers can assemble into quaternary complexes that resemble natural proteins in both solid-state structure and solution-phase stability. We note that our characterizations do not preclude some molten globule character of the Zwit-1F core in solution.[27] Nonetheless, these studies establish Zwit-1F as a remarkably protein-like stepping stone in the path toward fully synthetic mimics of biological molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Daniels DS, Petersson EJ, Qiu XJ, Schepartz A. J Am Chem Soc 2007;129:1532–1533. [PubMed: 17283998]
2. CD extrema between 205 and 215 nm have been attributed previously to the 3_{14}-helix. For details, see: Seebach D, Beck AK, Bierbaum DJ. Chem Biodiversity 2004;1:1111–1239.
3. See Supporting Information.
4. The MRE_{205} concentration dependence of Zwit-1F also fits a monomer–hexamer equilibrium, as did previous AU data. Although the Zwit-1F X-ray structure shows an octamer, we cannot currently exclude the presence of hexameric forms in solution.
5. Baxevanis AD, Godfrey JE, Moudrianakis EN. Biochemistry 1991;30:8817–8823. [PubMed: 1888742]
6. Daugherty MA, Brenowitz M, Fried MG. J Mol Biol 1999;285:1389–1399. [PubMed: 9917384]
7. Langerman NR, Klotz IM. Biochemistry 1969;8:4746–4757. [PubMed: 4312452]
8. Holmes MA, Stenkamp RE. J Mol Biol 1991;220:723–737. [PubMed: 1870128]
9. Nooren IMA, Thornton JM. J Mol Biol 2003;325:991–1018. [PubMed: 12527304]
10. Brooijmans N, Sharp KA, Kuntz ID. Proteins: Struct, Funct, Genet 2002;48:645–653. [PubMed: 12211032]
11. Tolan DR, Schuler B, Beernink PT, Jaenicke R. Biol Chem 2003;384:1463–1471. [PubMed: 14669989]
12. Wendt H, Baici A, Bosshard HR. J Am Chem Soc 1994;116:6973–6974.
13. Munson M, Balasubramanian S, Fleming KG, Nagi AD, Obrien R, Sturtevant JM, Regan L. Protein Sci 1996;5:1584–1593. [PubMed: 8844848]
14. Sturtevant JM. Annu Rev Phys Chem 1987;38:463–488.
15. Makhatadze GI, Privalov PL. Adv Protein Chem 1995;47:307–425. [PubMed: 8561051]
16. Dragan AI, Privalov PL. J Mol Biol 2002;321:891–908. [PubMed: 12206769]
17. Steif C, Weber P, Hinz HJ, Flossdorf J, Cesareni G, Kokkinidis M. Biochemistry 1993;32:3867–3876. [PubMed: 8471599]
18. Kirchhoff WM. NIST Technical Note 1993:1401.
19. Dragan AI, Li ZL, Makeyeva EN, Milgotina EI, Liu YY, Crane-Robinson C, Privalov PL. Biochemistry 2006;45:141–151. [PubMed: 16388589]
20. Makhatadze GI, Kim KS, Woodward C, Privalov PL. Protein Sci 1993;2:2028–2036. [PubMed: 7507751]
21. (a) Thompson KS, Vinson CR, Freire E. Biochemistry 1993;32:5491–5496. [PubMed: 8504069] See Supporting Information for comparison to GCN4 ΔH_{Cal} from (b) Dragan AI, Privalov PL. J Mol Biol 2002;321:891–908. [PubMed: 12206769]
22. Krishna MMG, Hoang L, Lin Y, Englander SW. Methods 2004;34:51–64. [PubMed: 15283915]

23. O'Shea EK, Lumb KJ, Kim PS. Curr Biol 1993;3:658–667. [PubMed: 15335856]

24. Lumb KJ, Kim PS. Biochemistry 1995;34:8642–8648. [PubMed: 7612604]

25. Glickson JD, Applequist J. J Am Chem Soc 1971;93:3276–3281. [PubMed: 5571295]

26. Schulman BA, Redfield C, Peng ZY, Dobson CM, Kim PS. J Mol Biol 1995;253:651–657. [PubMed: 7473740]

27. Although our DSC measurements support a two-state model, broad transitions of this type have been observed for proteins in molten globule states (Moosavi-Movahedi AA, Chamani J, Gharanfoli M, Hakimelahi GH. Thermochim Acta 2004;409:137–144. and designed proteins can become more structured in the solid state due to intermolecular crystal contacts Hill CP, Anderson DH, Wesson L, DeGrado WF, Eisenberg D. Science 1990;249:543. [PubMed: 2382133] A standard test for molten globule states is binding of the fluorophore ANS Goto Y, Fink AL. Biochemistry 1989;28:945–952. [PubMed: 2496758]), but this test is not feasible for Zwit-1F.[3] Experiments to examine the Zwit-1F structure using NMR relaxation techniques are in progress.

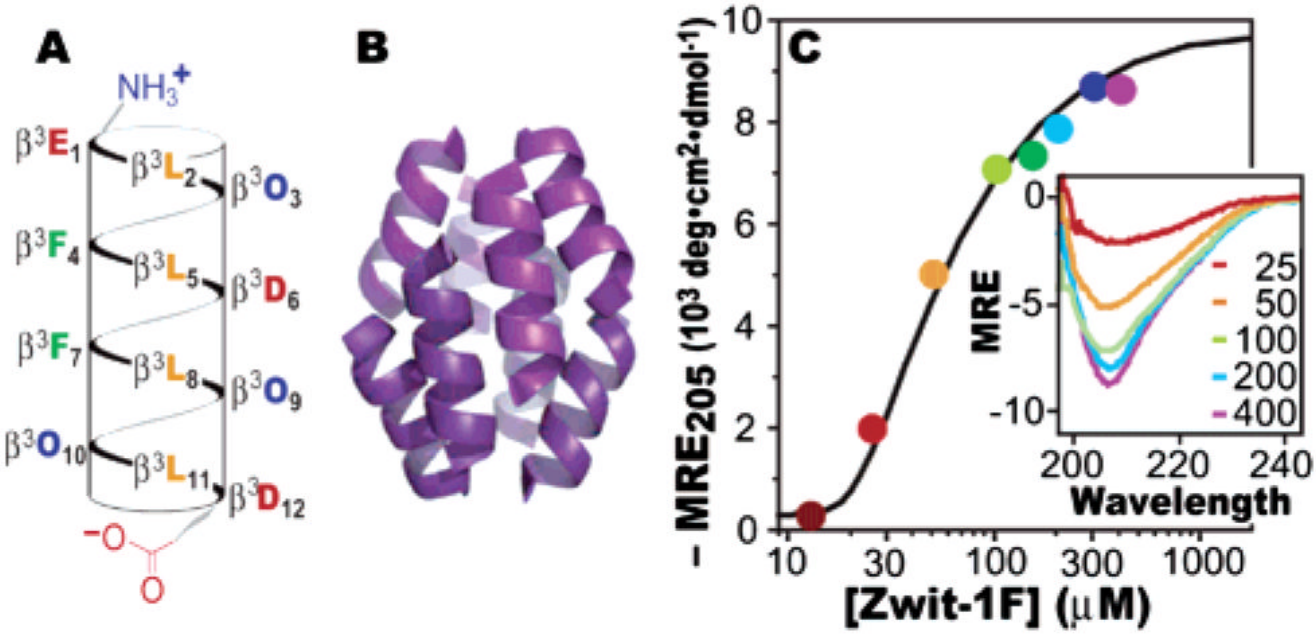

Figure 1.

(A) Helical net representation of the Zwit-1F monomer. β^3-Amino acids are designated by the single letter corresponding to the equivalent α-amino acid. O signifies ornithine. (B) Zwit-1F octamer structure determined by X-ray crystallography.[1] (C) Plot of MRE_{205} as a function of [Zwit-1F] fit to a monomer–octamer equilibrium. Inset: CD spectra (MRE in units of 10^3 deg·cm^2·dmol^{-1}) at the indicated [Zwit-1F] (μM).

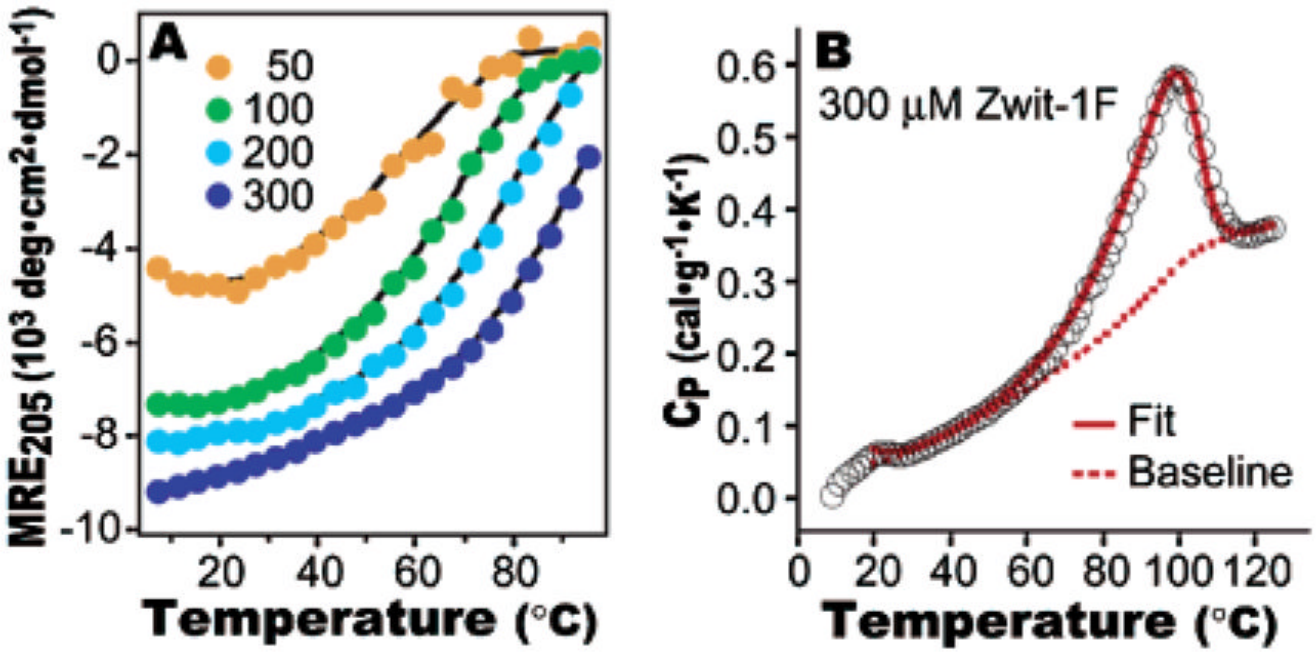

Figure 2.

(A) Temperature-dependent CD analysis of Zwit-1F. Plot of MRE_{205} as a function of temperature at the indicated Zwit-1F concentration (μM). (B) DSC analysis of Zwit-1F unfolding fit to a subunit dissociation model. Raw data are shown as black circles.[3]

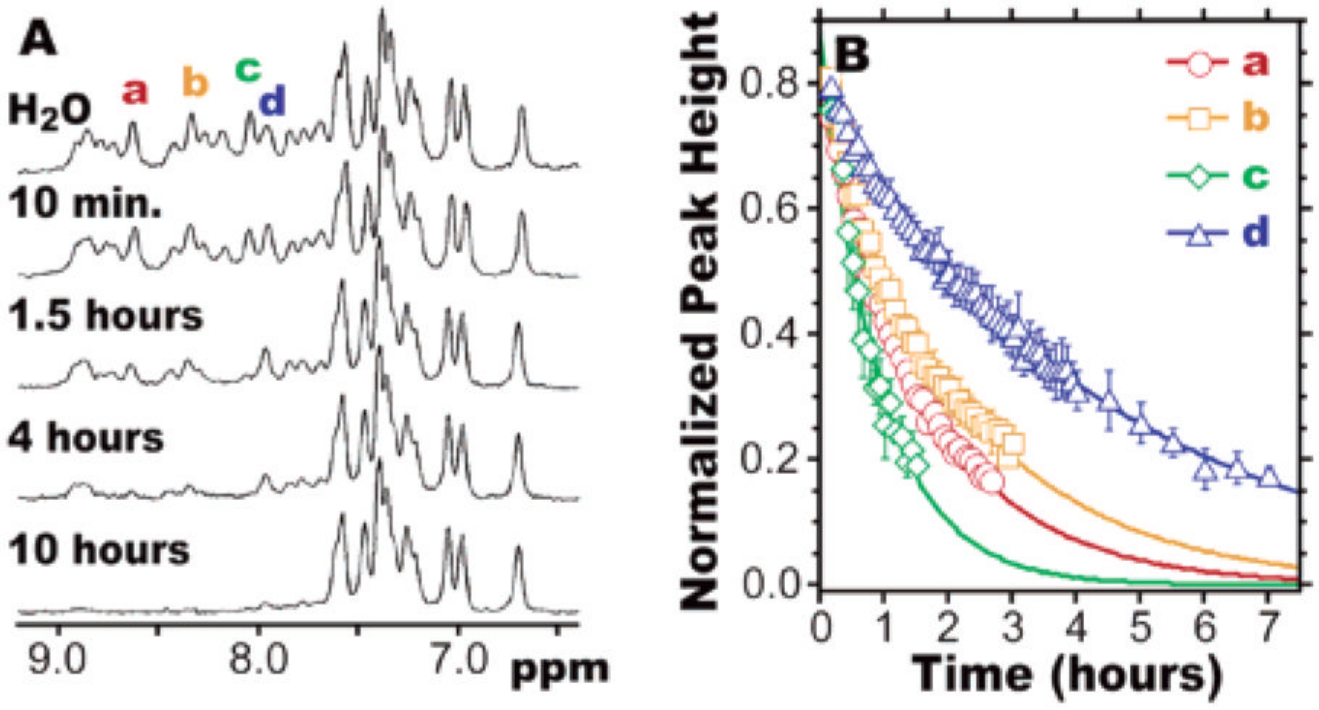

Figure 3.

(A) 500 MHz ^{1}H NMR spectra of 1.5 mM Zwit-1F, acquired in phosphate-buffered "H_2O" (9:1 H_2O/D_2O) or at the indicated times after reconstitution of a lyophilized Zwit-1F sample in phosphate-buffered D_2O. (B) Peak heights of the indicated resonances (normalized to the peak at 6.70 ppm) fit to exponential decays.[3] Bars indicate standard error.

Table 1

Comparison of Protein Association Parameters[a]

protein (stoichiometry)	$MW_{monomer}$	ΔG_{area}
Zwit-1F (8)	1.6 kDa	5.9
hemerythrin (8)	13.8 kDa	$_{3.3}7$
aldolase (4)	39.2 kDa	$_{3.9}11$
GCN4 (2)	4.0 kDa	$_{4.8}12$
ROP (2)	7.2 kDa	$_{\geq 3.0}13$

[a] ΔG_{area} values in units of cal· mol^{-1}·$Å^{-2}$. Interaction surface areas and ΔG_{area} calculated as described in Supporting Information.